

CLAIMS

What is claimed is:

5 1. A truncated glial cell line-derived neurotrophic factor (GDNF) protein product having an amino acid sequence

X-[Cys⁴¹-Cys¹³³]-Y

wherein

[Cys⁴¹-Cys¹³³] represents the amino acid sequence of Cys⁴¹ through 10 Cys¹³³ as depicted in Figure 1 (SEQ ID NO 2);

Y represents the carboxy terminal group of Cys¹³³ or a carboxy-terminus amino acid residue of Ile¹³⁴; and

X represents a methionylated or nonmethionylated amine group of Cys⁴¹ or amino-terminus amino acid residue(s) selected from the group:

15

G	
RG	
NRG	
KNRG (SEQ ID NO:3)	
GKNRG (SEQ ID NO:4)	
RGKNRG (SEQ ID NO:5)	
QRGKNRG (SEQ ID NO:6)	
GQRGKNRG (SEQ ID NO:7)	
RGQRGKNRG (SEQ ID NO:8)	
RRGQRGKNRG (SEQ ID NO:9)	
G RRGQRGKNRG (SEQ ID NO:10)	
KG RRGQRGKNRG (SEQ ID NO:11)	
GKG RRGQRGKNRG (SEQ ID NO:12)	
RGKG RRGQRGKNRG (SEQ ID NO:13)	
SRGKG RRGQRGKNRG (SEQ ID NO:14)	
NSRGKG RRGQRGKNRG (SEQ ID NO:15)	
ENSRGKG RRGQRGKNRG (SEQ ID NO:16)	
PENSRGKG RRGQRGKNRG (SEQ ID NO:17)	
NPENSRGKG RRGQRGKNRG (SEQ ID NO:18)	
ANPENSRGKG RRGQRGKNRG (SEQ ID NO:19)	
A ANPENSRGKG RRGQRGKNRG (SEQ ID NO:20)	

	AA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:21)
	AAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:22)
	QAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:23)
	RQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:24)
	NRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:25)
	RNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:26)
	ERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:27)
	RERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:28)
	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:29)
P	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:30)
LP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:31)
VLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:32)
AVLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:33)
MAVLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:34)
QMAVLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:35)
KQMAVLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:36)
DKQMAVLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:37) and
PDKQMAVLP	RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:38)

and addition, substitution and internal deletion variants and derivatives thereof.

2. A truncated GDNF protein product according to Claim 1, wherein X =
- 5 RQAAA ANPENSRGKG RRGQRGKNRG (SEQ ID NO:24) or a variant thereof.
3. A truncated GDNF protein product according to Claim 1, wherein X =
- 10 NPENSRGKG RRGQRGKNRG (SEQ ID NO:18) or a variant thereof.
- 15 4. A truncated GDNF protein product according to Claim 1, wherein X =
- PENSRGKG RRGQRGKNRG (SEQ ID NO:17) or a variant thereof.
5. A truncated GDNF protein product according to Claim 1, wherein X =
- SRGKG RRGQRGKNRG (SEQ ID NO:14) or a variant thereof.
- 15 6. A truncated GDNF protein product according to Claim 1, wherein X =
- RGQRGKNRG (SEQ ID NO:8) or a variant thereof.

7. A truncated GDNF protein product according to Claim 1, wherein X = GQRGKNRG (SEQ ID NO:7) or a variant thereof.

8. A truncated GDNF protein product according to Claim 1, wherein X = 5 KNRG (SEQ ID NO:3) or a variant thereof.

9. A truncated GDNF protein product according to Claim 1, wherein X = NRG or a variant thereof.

10 10. A truncated GDNF protein product according to Claims 1 through 9, wherein said amino acid sequence is glycosylated.

11. A truncated GDNF protein product according to Claims 1 through 9, wherein said amino acid sequence is nonglycosylated.

15 12. A truncated GDNF protein product according to Claim 1, wherein said derivative is an X-[Cys⁴¹-Cys¹³³]-Y amino acid sequence conjugated to a water soluble polymer.

20 13. A polynucleotide encoding a truncated GDNF protein according to Claim 1.

14. A polynucleotide according to Claim 13, comprising a portion of the sequence as set forth in Figure 1.

25 15. A polynucleotide according to Claim 13, comprising a portion of the sequence as set forth in Figure 3.

16. A polynucleotide according to Claim 13, comprising a portion of the sequence as set forth in Figure 4.

30 17. A polynucleotide according to Claim 13, comprising the sequence as set forth in Figure 5.

18. A polynucleotide according to Claim 13, comprising the sequence as set 35 forth in Figure 6.

19. A polynucleotide according to Claim 13, comprising the sequence as set forth in Figure 7.

20. A vector, comprising a polynucleotide of Claim 13 operatively linked to an expression control sequence.

21. A prokaryotic or eukaryotic host cell transformed or transfected with a polynucleotide of Claim 13.

22. A method for producing a truncated GDNF protein, comprising growing host cells of Claim 21 in a suitable nutrient medium and, optionally, isolating said truncated GDNF from said cells or said nutrient medium.

23. A method for producing a truncated GDNF protein according to Claim 22, wherein said host cells are *E. coli*.

24. A method for producing a truncated GDNF protein according to Claim 22, wherein said host cells are Chinese hamster ovary cells.

25. A method for the production of a truncated glial cell line-derived neurotrophic factor (GDNF) protein, comprising the steps of:

- culturing a prokaryotic or eukaryotic host cell transformed or transfected with a vector of Claim 20;
- maintaining said host cell under conditions allowing the expression of truncated GDNF protein by said host cell; and
- optionally isolating the truncated GDNF protein expressed by said host cell.

26. A truncated GDNF protein which is the recombinant expression product of a prokaryotic or eukaryotic host cell containing an exogenous polynucleotide of Claim 13.

27. A pharmaceutical composition comprising a truncated GDNF protein product according to Claim 1 in association with a pharmaceutically acceptable vehicle.

28. A pharmaceutical composition comprising a truncated GDNF protein, produced in accordance with the method of Claim 22 in association with a pharmaceutically acceptable vehicle.

5 29. A pharmaceutical composition comprising a truncated GDNF protein, produced in accordance with the method of Claim 25 in association with a pharmaceutically acceptable vehicle.

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30. A method of treating Parkinson's Disease comprising administering to a patient the pharmaceutical composition of Claim 27.

15 31. A method of treating Parkinson's Disease comprising administering to a patient a polynucleotide sequence of Claim 13 to provide *in vivo* production of said truncated GDNF protein.

32. A method of treating Parkinson's Disease comprising implanting in a patient a cell transformed with a polynucleotide sequence of Claim 13 to provide *in vivo* production of said truncated GDNF protein.

20 33. A glial cell line-derived neurotrophic factor (GDNF) composition, comprising a mature GDNF protein and one or more truncated GDNF proteins, wherein said mature GDNF protein has a molecular weight of approximately 44 kDa, and wherein said truncated GDNF protein(s) has a molecular weight of approximately 29 to 40 kDa.

25 34. A GDNF composition according to Claim 33, comprising at least two species of truncated GDNF protein wherein a first species has a molecular weight of approximately 36 kDa and a second species has a molecular weight of approximately 40 kDa.

30 35. A GDNF composition according to Claim 34, wherein said second truncated GDNF species having a molecular weight of approximately 40 kDa is a heterodimer of a mature GDNF monomer having a molecular weight of approximately 22 kDa and a truncated GDNF monomer having a molecular weight of approximately 18 kDa.

36. A truncated GDNF protein isolated from the GDNF composition of Claim 33 and having a molecular weight of approximately 29 to 40 kDa.

37. A truncated GDNF protein isolated from the GDNF composition of 5 Claim 33 and having a molecular weight of approximately 29 to 36 kDa.

38. A truncated GDNF protein derived from a mature GDNF protein expressed by a recombinantly modified bacterial or mammalian cell, said truncated GDNF protein having an amino acid sequence

10 X-[Cys⁴¹-Cys¹³³]-Y

wherein

[Cys⁴¹-Cys¹³³] represents the amino acid sequence of Cys⁴¹ through Cys¹³³ as depicted in Figure 1 (SEQ ID NO 2);

15 Y represents the carboxy terminal group of Cys¹³³ or a carboxy-terminus amino acid residue of Ile¹³⁴; and

X represents an amine group of Cys⁴¹ or amino-terminus amino acid residue(s) selected from the group:

	G
	RG
	NRG
	KNRG (SEQ ID NO:3)
	GKNRG (SEQ ID NO:4)
	RGKNRG (SEQ ID NO:5)
	QRGKNRG (SEQ ID NO:6)
	GQRGKNRG (SEQ ID NO:7)
	RGQRGKNRG (SEQ ID NO:8)
	RRGQRGKNRG (SEQ ID NO:9)
G	RRGQRGKNRG (SEQ ID NO:10)
KG	RRGQRGKNRG (SEQ ID NO:11)
GKG	RRGQRGKNRG (SEQ ID NO:12)
RKG	RRGQRGKNRG (SEQ ID NO:13)
SRKG	RRGQRGKNRG (SEQ ID NO:14)
NSRGKG	RRGQRGKNRG (SEQ ID NO:15)
ENSRKG	RRGQRGKNRG (SEQ ID NO:16)
PENSRKG	RRGQRGKNRG (SEQ ID NO:17)

	NPENSRGKG	RRGQRGKNRG (SEQ ID NO:18)
	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:19)
A	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:20)
AA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:21)
AAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:22)
QAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:23)
RQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:24)
NRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:25)
RNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:26)
ERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:27)
RERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:28)
RRERNRQAAA	ANPENSRGKG	RRGQRGKNRG (SEQ ID NO:29)
P	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:30)
LP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:31)
VLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:32)
AVLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:33)
MAVLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:34)
QMAVLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:35)
KQMAVLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:36)
DKQMAVLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:37) and
PDKQMAVLP	RRERNRQAAA	ANPENSRGKG RRGQRGKNRG (SEQ ID NO:38)

and addition, substitution and internal deletion variants thereof.

39. A truncated GDNF protein according to Claim 38, wherein X is selected
5 from the group consisting of:

3
RG
NRG
KNRG (SEQ ID NO:3)
GKNRG (SEQ ID NO:4)
RGKNRG (SEQ ID NO:5)
QRGKNRG (SEQ ID NO:6)
GQRGKNRG (SEQ ID NO:7)
RGQRGKNRG (SEQ ID NO:8) and
RRGQRGKNRG (SEQ ID NO:9)

and variants thereof.

40. A truncated GDNF protein according to Claim 38, wherein mature GDNF protein is expressed by a recombinantly modified bacterial cell and said

5 truncated GDNF protein is produced *in vitro* or *in vivo*.

41. A method of preparing a pharmaceutical composition wherein a therapeutically effective amount of a truncated GDNF protein product according to Claim 1 is mixed with one or more pharmaceutically acceptable vehicles.

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42. The use of a truncated GDNF protein product according to Claim 1 for treating damage to the nervous system caused by disease or injury.

43. The use of a truncated GDNF protein product according to Claim 42 for

15 treating Parkinson's disease.

44. The use of a truncated GDNF protein product according to Claim 1 for the preparation of a pharmaceutical composition for treating damage to the nervous system caused by disease or injury.

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